PROTOCOL

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Reverse phase cartridge desalting of RNA oligonucleotides

This protocol is for the reverse phase cartridge desalting of RNA oligonucleotides.

Materials required

Consumables

1. Qty. 1—C18 reversed phase cartridge (Waters SepPak Cat #020515)
2. Qty. 1—10 mL syringe with luer tip
3. Qty. 2—15 mL conical tubes
4. Qty. 5—1.7 mL Microfuge tubes

Chemicals

1. Acetonitrile (MeCN) (10 mL)
2. 50% MeCN : 50% 100 mM Sodium Acetate (NaOAc) (10 mL)
3. 100 mM NaOAc (15 mL)
4. 10 mM Triethylammonium bicarbonate (TEAB) (See notes below for preparation) (10 mL)
5. Elution Buffer: 35% MeCN : 35% Methanol (MeOH) 30% : 10 mM TEAB (5 mL)

Procedured

Equilibrate cartridge

1. Attach a SepPak cartridge to the tip of the 10 mL syringe with the plunger removed.
2. Add 10 mL MeCN to the syringe and slowly depress the plunger to wash the MeCN through the cartridge dropwise, 2-3 drops/second.
3. Remove the syringe from the cartridge and then remove the plunger. Reattach the SepPak cartridge to the tip of the syringe in the same orientation. Fill the syringe with 10 mL 50% MeCN : 50% 100 mM NaOAc. Again wash the buffer through the cartridge dropwise, 2-3 drops/second.
4. Remove the cartridge and then the plunger. Reattach the cartridge, fill the syringe with 10 mL, 100 mM NaOAc, and wash the buffer through the cartridge dropwise, 2-3 drops/second.

Load sample

1. Bring the RNA oligonucleotide up in 5 mL of 100 mM NaOAc.
2. Remove the cartridge and plunger from the syringe and then reattach the cartridge to the syringe. Add the oligonucleotide solution to the syringe.
3. Collect the drops, 1-2 per second, from the cartridge in a 15 mL conical. **(Note: It is recommended that this step be performed very slowly to ensure that the oligonucleotides bind to the C18 resin in the cartridge.)** Save this conical.

Wash

1. Remove the cartridge and plunger. Reattach the cartridge and add 10 mL of 10 mM TEAB.
2. Wash through the cartridge dropwise, 2-3 drops/second. Collect this fraction in 15 mL conical tube and save.

Elute

1. Remove the cartridge from the syringe and then remove the plunger. Reattach the cartridge and position over a 1.7 mL microfuge tube. Add 5 mL elution buffer to the syringe. Collect ~1 mL fractions in five microfuge tubes.
2. The majority of the oligonucleotide should be in the first two elution fractions. Check for the oligonucleotide in all fractions (Load, Wash, and all elutions) by measuring the absorbance at 260 nm on a UV spectrophotometer.
3. Dry down the elution aliquot(s) containing the oligonucleotide, and resuspend as required for the subsequent application.

Notes

1. The capacity of the SepPak is 75 OD260 units (ODU). A larger SepPak is available (Cat #023635) which can accommodate 150 ODUs. The identical protocol is used with the larger SepPak.
2. The RNA binds more efficiently when loaded in a salt buffer. In this protocol 100 mM NaOAc is used.
3. As TEAB is volatile, it is used in the wash and in the elution buffers so that the eluted RNA oligo will be salt-free after drying down under vacuum. If desired, other salts may be substituted.
4. Preparation of 500 mL 2 M TEAB stock solution: Add 139 mL triethylamine to 325 mL sterile water. Two phases will form. Bubble carbon dioxide through the solution with a bubbler while stirring. This may be accomplished by placing dry ice in a closed container with tubing connected to the bubbler. As the dry ice sublimates the carbon dioxide gas will be forced through the bubbler into the TEAB solution. Continue bubbling until the pH is less than 8.0. Bring volume up to 500 mL with sterile water. Store tightly sealed at 4 °C.

If you have any questions, contact

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